ENVIRONMENTALLY-SENSITIVE VESICLES FOR CONTROLLED DRUG DELIVERY

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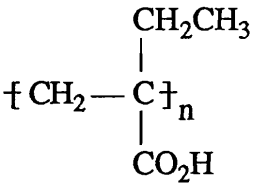
A. INTRODUCTION

The potential uses of phospholipid vesicles in drug delivery have been widely discussed.[1-7] The present chapter summarizes work from this laboratory on the preparation of lipid vesicles that release their contents in response to specific chemical or physical stimuli.[8-14] Contributions to this area from other laboratories may be found in references 15-21.

The unifying theme of our work has been the use of environmentally sensitive polyelectrolytes to control the structure and permeability of the phospholipid vesicle membrane. In particular, we have found that the large changes in solvation and conformation that accompany the titration of hydrophobic poly(carboxylic acid)s can be exploited to effect phospholipid reorganization from vesicular to micellar form, as illustrated in Scheme I. This idea has been elaborated, as described below, to prepare phospholipid vesicles that respond to changes in pH, temperature or glucose concentration.

B. GENERAL CONSIDERATIONS

Most of our work to date has concerned the interactions of poly(2-ethylacrylic acid) (PEAA, 1) with natural or synthetic phosphatidylcholines (2). PEAA in water undergoes a sharp conform-



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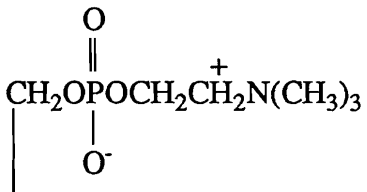

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ational transition near physiological pH, from an expanded, hydrophilic coil in basic solutions to a compact globule upon acidification.[22-24] The same changes in water-polymer interactions that cause conformational collapse also force the PEAA chain to adsorb strongly to the lipid membrane (and presumably to other surfaces as well). Reorganization of the bilayer occurs in order to

accommodate the adsorbed chains, with the result that the preferred packing arrangement in the mixed polymer-lipid aggregate is micellar, rather than vesicular. The conversion from vesicles to micelles compromises the barrier properties of the vesicular membrane, and allows rapid, quantitative release of contents. In our experience, PEAA is virtually unique among synthetic polyelectrolytes in providing high sensitivity to pH in the physiological range, and in causing membrane reorganization without complications arising from vesicular aggregation. Although PEAA is not available from commercial sources, its preparation is not difficult. Detailed procedures for the preparation of PEAA are available from the author.

The phosphatidylcholines serve as a convenient source of pure, bilayer-forming surfactants of systematically variable structure, and their physical properties are relatively well understood. In addition, the phosphatidylcholines are the predominant lipids in the outer monolayer of mammalian cell membranes; they serve, therefore, as a logical starting point for the construction of non-toxic, non-immunogenic vesicular delivery systems.

C. CONJUGATION OF PEAA TO PHOSPHATIDYLCHOLINE SURFACES

Therapeutic uses of the membrane reorganization process shown in Scheme 1 will require that the polyelectrolyte remain irreversibly bound to the bilayer and that the average systemic concentration of free polyelectrolyte remain vanishingly small. In principle, this can be achieved via surface conjugation of the polyelectrolyte. The procedure that we use for surface conjugation of PEAA is shown in Scheme 2,[12] and is based on the method of Martin and Papahadjopoulos.[25]

The conjugation method relies on the Michael addition of polymer-bound thiol groups to a small number of N-alkylmaleimide functions introduced to the membrane surface in the form of the modified phospholipid, dimyristoyl N-[(4-maleimidomethyl)cyclohexanecarbonyl]phosphatidylethanolamine (Mal-PE, 3). Mal-PE is readily prepared in a single step from dimyristoylphosphatidylethanolamine and succinimidyl 4-maleimidomethylcyclohexane carboxylate (SMCC, available from Aldrich Chemical Co.). Hydration of a 9:1 mixture of egg phosphatidylcholine and 3,

followed by sonication of the hydrated mixture, affords vesicle suspensions characterized by size distributions virtually identical to those obtained from the pure phosphatidylcholine.

Thiolation of PEAA is similarly straightforward. Carbodiimide-promoted coupling of polymer-bound carboxyl groups with 2-aminoethanethiol allows routine introduction of 1-3 mol-% of thiol functionality without any apparent competitive formation of the isomeric aminothioester. The polymer-bound thiols are subject to reversible oxidative crosslinking, and so are reduced with dithiothreitol (DTT) immediately prior to use. DTT is then readily removed from the polymer by chromatography on Sephadex G-25.

Attachment of PEAA to the surface of egg phosphatidylcholine vesicles can be accomplished by incubation of the thiolated polymer with vesicles prepared from a 9:1 mixture of the phosphatidylcholine and 3. Free polyelectrolyte chains are separated from the vesicular preparation by chromatography on Sepharose CL-6B. Typical yields of surface-bound PEAA are of the order of 50 μg of polymer bound per mg of phospholipid.

The sensitivity of such vesicle preparations to small changes in pH may be demonstrated by an experiment in which the fluorescent dye, calcein, is entrapped in the vesicle interior at pH 7.0. Vesicle loading at a dye concentration of 200 mM leads to quenching of the calcein fluorescence, so that release of vesicle contents can be detected by an increase in emission as the quenching condition is relieved.

Figure 1 shows the results of such an experiment, in which the surface-loading of PEAA is ca. 50 μg/mg of egg phosphatidylcholine. At room temperature and pH 7.0, the fluorescence intensity remains constant over a period of 10 min. Acidification of the suspension to pH 6.5 then causes a rapid increase in emission intensity, as the contents of the vesicles are released quantitatively. The effectiveness of the polyelectrolyte is remarkable, given the modest concentration of polymer on the membrane surface.

Sensitivity to pH in this range (7.4-6.5) is potentially useful in several areas of drug delivery, as a result of the fact that conditions of abnormal acidity may arise in inflamed or infected areas, in certain tumor tissues, or in ischemia. Papahadjopoulos and coworkers26 have also suggested that pH-sensitization may be generally useful in vesicular delivery systems, in that it provides a mechanism for rapid release into the cytoplasm of drugs entrapped in liposomes that have been taken up by cells via endocytosis. Such liposomes undergo acidification after uptake, but highly charged or high molecular weight species gain access to the cytoplasm only very slowly. A pH trigger would promote rapid release to the cytoplasm after endocytic uptake. The precise pH-response of PEAA-modified vesicles can be optimized for a particular application through variations in polymer molecular weight, composition and stereochemistry.[8,13]

D. TEMPERATURE-SENSITIZATION

The rate of the polyelectrolyte-driven reorganization of phosphatidylcholine membranes is dependent on temperature, and passes through a maximum at the main melting transition of the lipid bilayer. This provides a potential basis for the development of temperature-sensitive vesicles for use in delivery of therapeutic agents to targets characterized by local hyperthermia.

Figure 2 shows results for dipalmitoylphosphatidylcholine (DPPC), which melts at 41°C. Hydration of DPPC followed by room-temperature addition of a 1 mg/ml solution of PEAA at pH 6.5, affords a stable, turbid vesicular dispersion. On warming through the phase transition, however, the turbidity of the suspension is rapidly lost as a consequence of the PEAA-driven vesicle-to-micelle transition. Although we have not demonstrated temperature-dependent release of the contents of vesicles prepared in this way, it is virtually certain that rapid, quantitative release would be observed at the clarification temperature. Of course, the intrinsic permeability of pure lipid bilayers increases substantially at the phase transition temperature as well.[27] We have not conducted experiments analogous to that shown in Figure 2 with vesicles bearing surface-conjugated PEAA.

E. GLUCOSE-DEPENDENT MEMBRANE REORGANIZATION

Scheme 3 illustrates a general approach to the formulation of phospholipid vesicles that release their contents in response to elevated concentrations of organic solutes of physiological interest. The approach uses an enzyme - or perhaps a cascade of enzymes - to convert the species of interest into a source of H+. The elevated local concentration of H^+ is then exploited as described above to effect membrane reorganization and release of vesicle contents.

A particularly intriguing example of this approach employs the enzyme glucose oxidase to provide H^+ in proportion to the local concentration of glucose.[10] Hydration of phosphatidylcholines in mixed solutions of PEAA and glucose oxidase affords vesicular dispersions. Addition of physiological concentrations of glucose to such preparations causes a depression of pH and concommitant membrane reorganization and loss of turbidity. Figure 3 shows a typical time course for the turbidity loss. The implications of this approach in the development of self-regulated insulin delivery systems are clear, and generalization to other substrates of medical interest is readily imagined.

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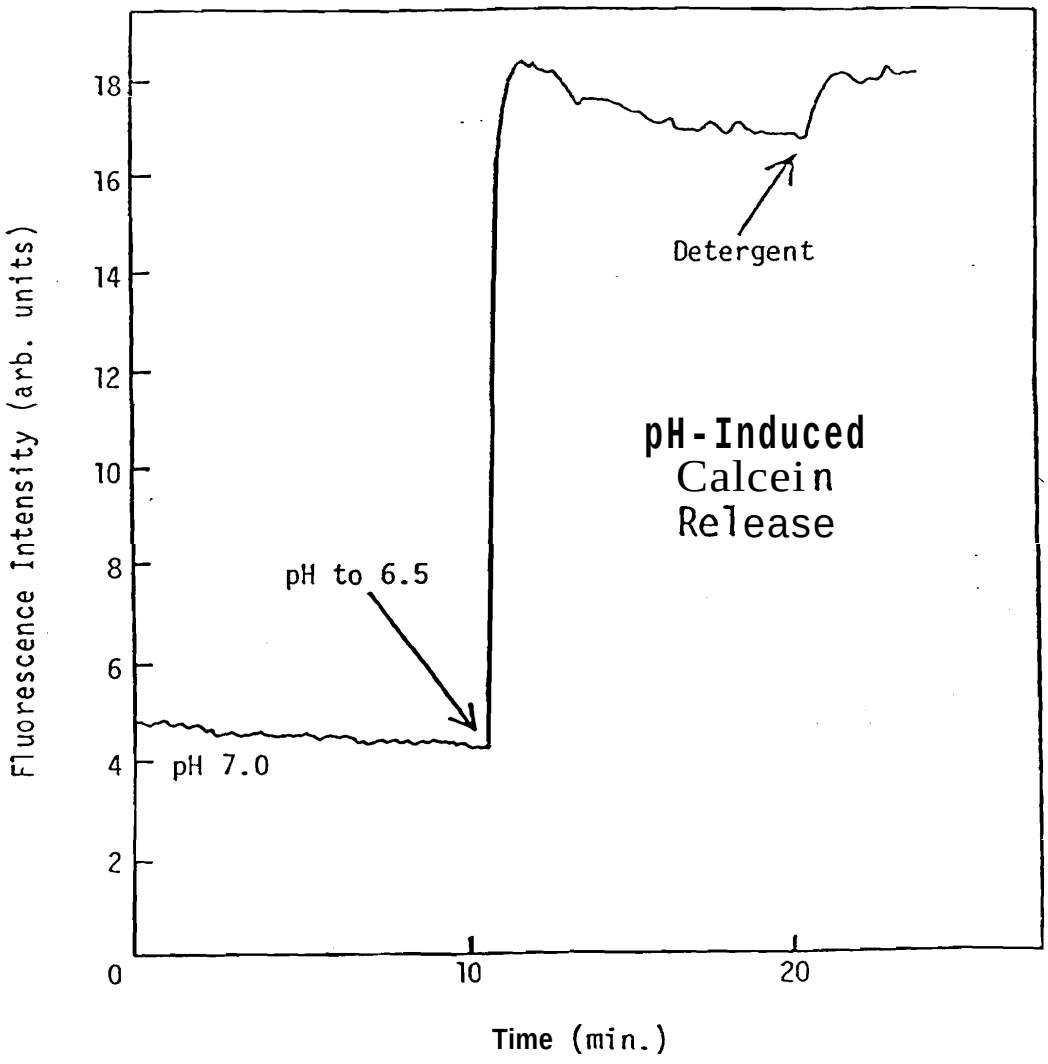
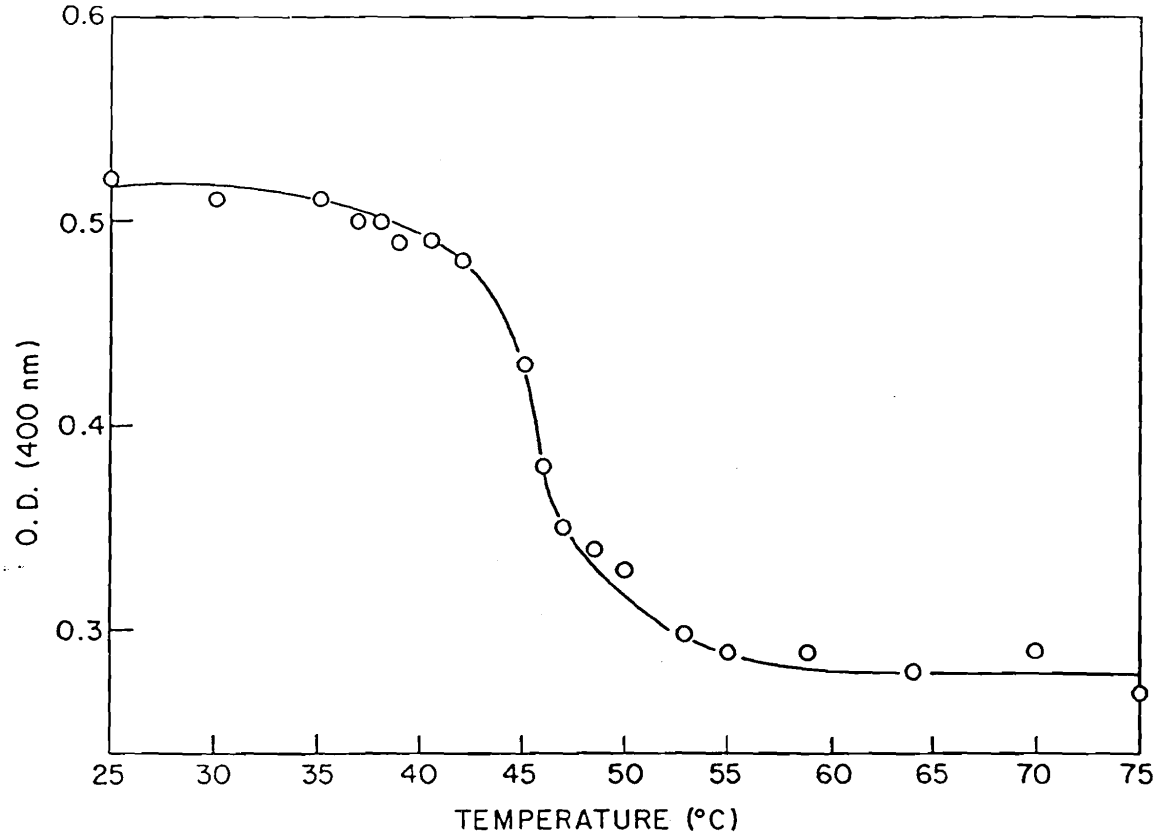
CAPTIONS FOR FIGURES

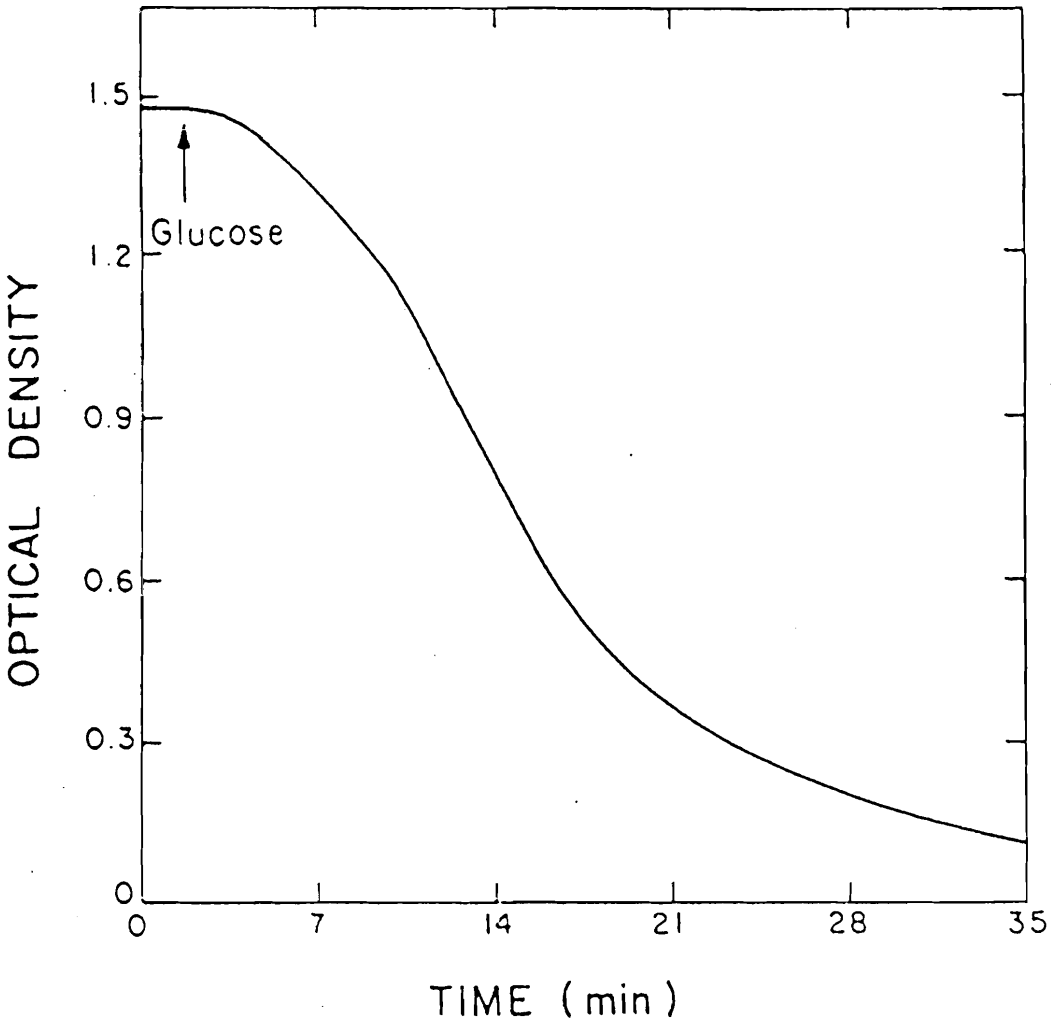
Figure 1. Time-dependent fluorescence emission from vesicle-entrapped calcein. Dye was entrapped at a concentration of 200 mM in vesicles prepared from a 9:1 mixture of egg phosphatidylcholine and 3. **PEAA** was conjugated to the vesicle surface to a level of ca. 50 μg **PEAA per** mg lipid. Initial pH of 7.0 was depressed to 6.5 after 10 min by addition of ca. 5 μL of 1N HCl.

Figure 2. Optical density (400 nm) of a multilamellar suspension of **DPPC** in 0.1% aqueous **PEAA,** pH 6.5, as a function of temperature.

Figure 3. Optical density (400 nm) of an aqueous suspension of dilauroylphosphatidylcholine (2.4 mg/mL), **PEAA** (2.6 mg/mL) and glucose oxidase (0.7 mg/mL), prior and subsequent to addition of glucose (1.3 mg/mL). Arrow marks time of glucose addition.


Fluorescence Intensity (arb. units)
18
16
14
12
10
8
6
4
2
0
10
20
Time (min.)
pH 7.0
pH to 6.5
Detergent
pH-Induced
Calcein
Release


0.6
0.5
0.4
0.3
O. D. (400 nm)
25
30
35
40
45
50
55
60
65
70
75
TEMPERATURE (°C)

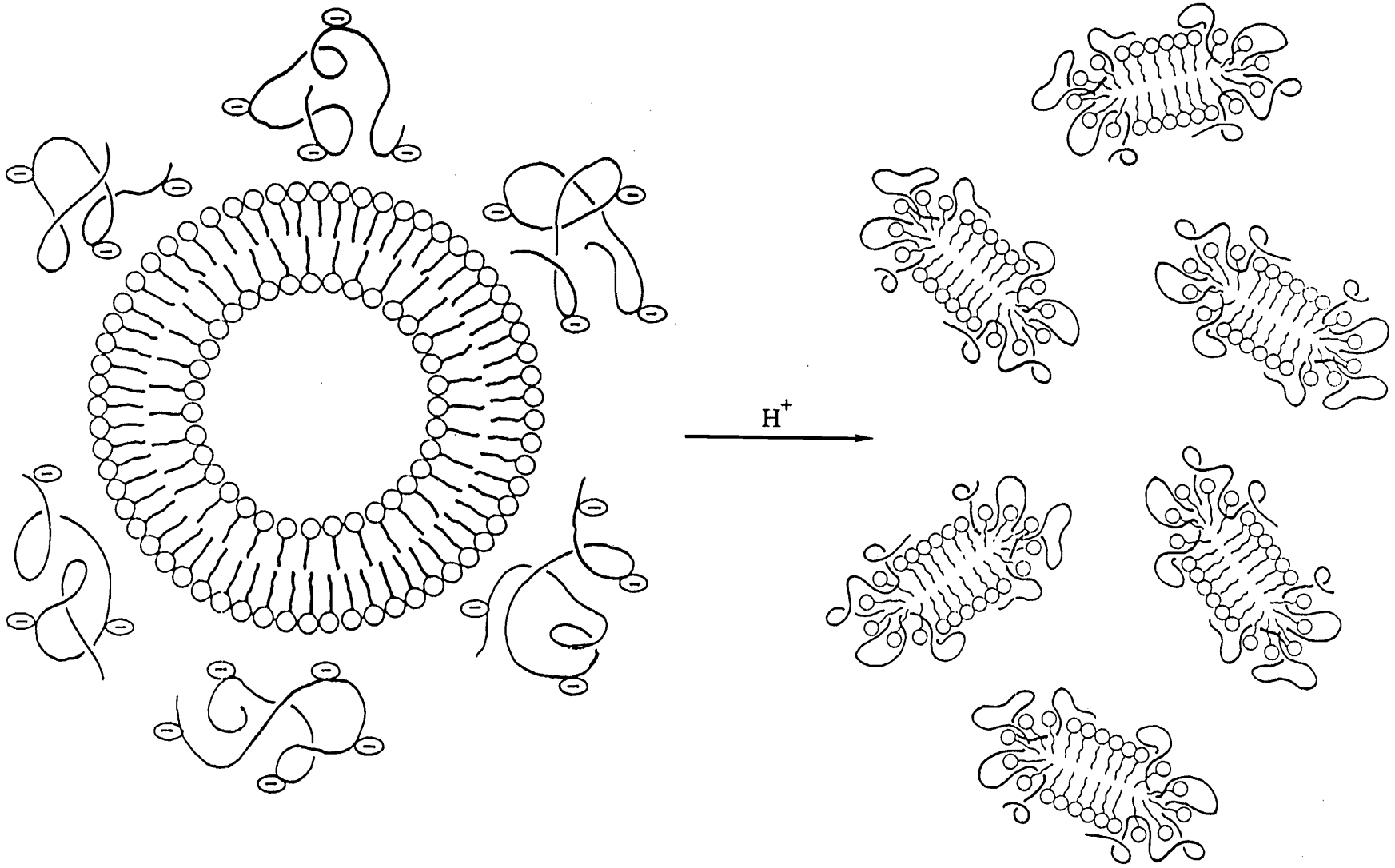

OPTICAL DENSITY
1.5
1.2
0.9
0.6
0.3
0
Glucose
0
7
14
21
28
35
TIME (min)

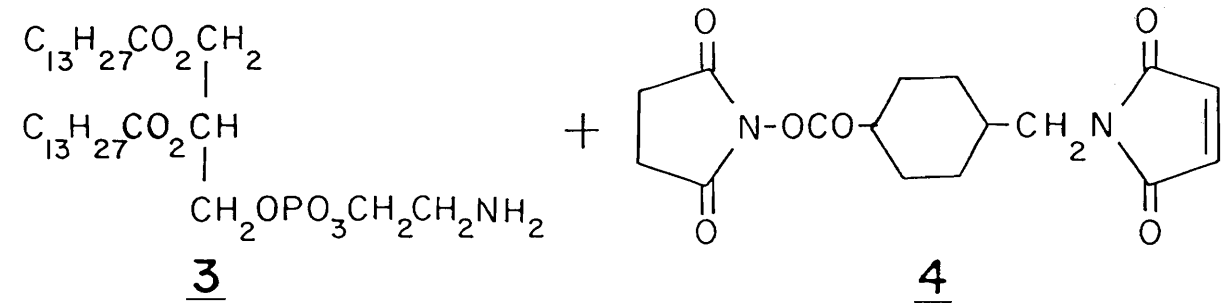
CAPTIONS FOR SCHEMES

Scheme 1. **pH-Dependent reorganization of phospholipid vesicle membranes by PEAA.**

Scheme 2. **Conjugation of PEAA to phosphatidylcholine membrane surfaces.**

Scheme 3. **Use of oxidative or hydrolytic enzymes to prepare vesicular membranes sensitive to organic solutes.**


H+



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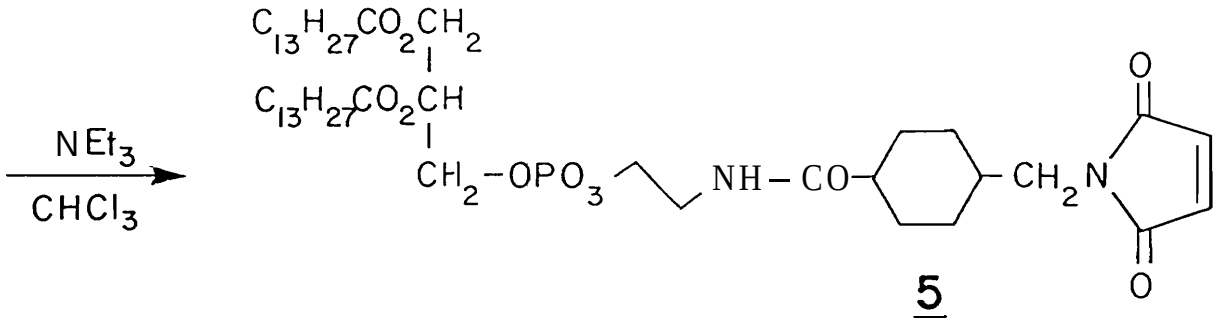

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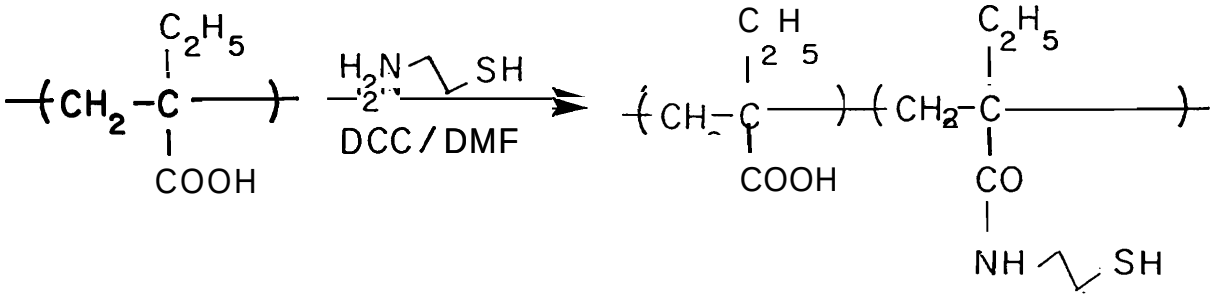

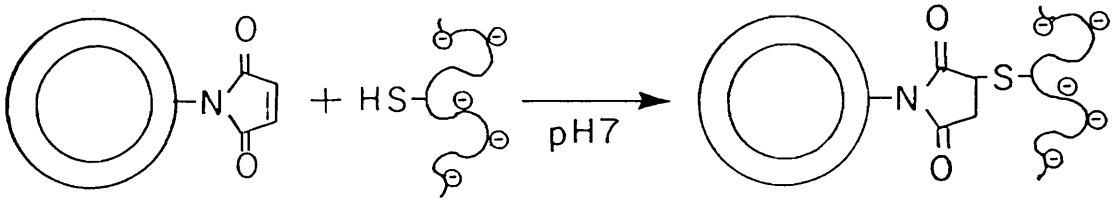

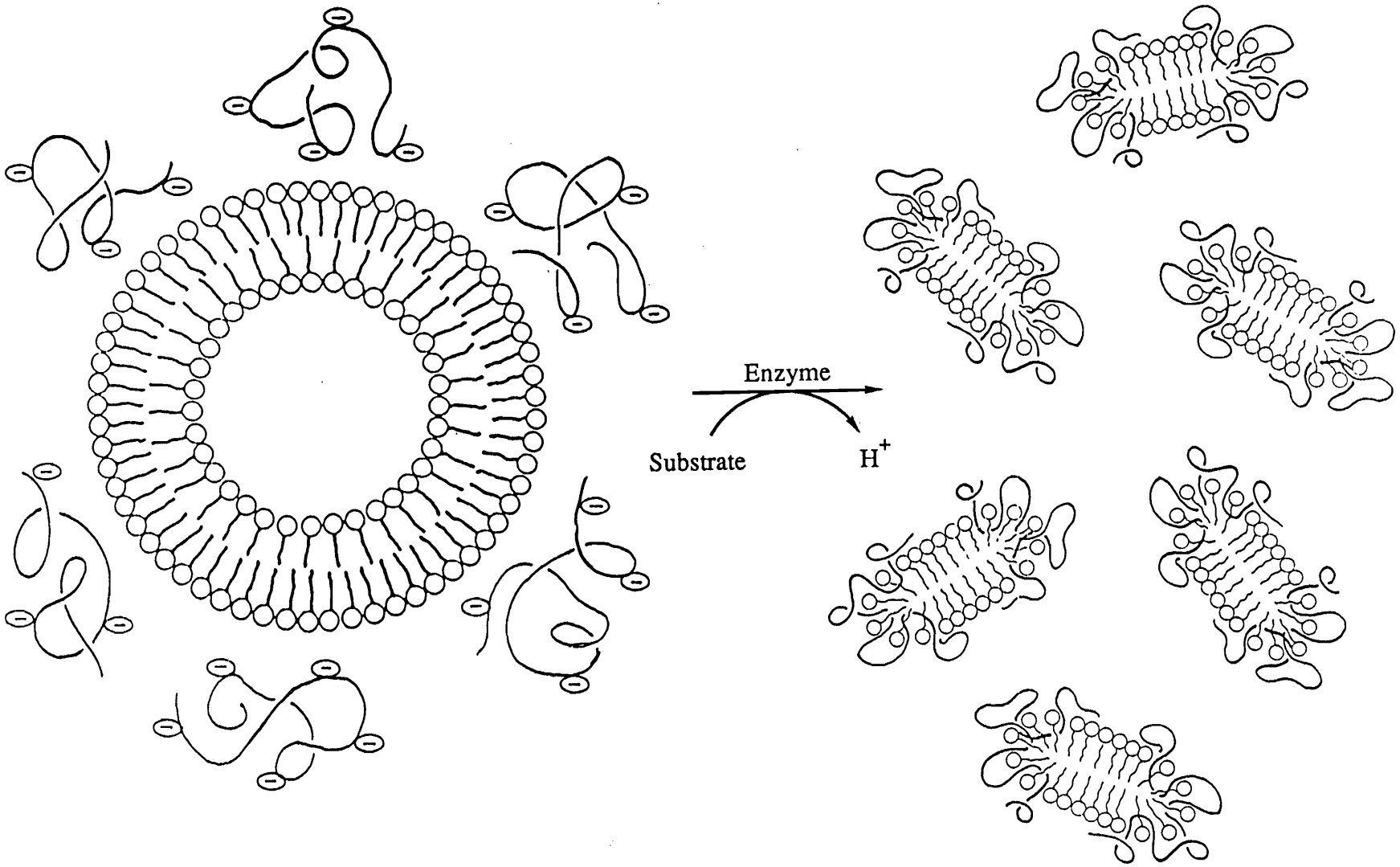
Enzyme
Substrate
H+